43. (New) A method for raising an antigen response in an animal against at least one biological activity of a wild type SPE-C comprising: administering an immunogenic composition according to claim 42 to an animal.

44. (New) A method for reducing symptoms associated with toxic shock comprising: administering an immunogenic composition according to claim 42 to an animal.

Remarks

Applicants have received and reviewed the Office Action mailed February 8, 2001. By way of response, Applicants have cancelled claims 1, 3-12, and 15-23 without prejudice and added claims 24-44. Claims 24-44 are now pending. No new matter is introduced. Applicants submit that the newly presented claims are supported by the specification.

Applicants appreciate and acknowledge the withdrawal of the rejections under 35 U.S.C. § 103(a).

For the reasons given below, Applicants respectfully submit that the newly presented claims are in condition for allowance, and notification to that effect is earnestly solicited.

35 USC § 112, First Paragraph Rejections

The Examiner rejected claims 1, 3-12, and 15-23 based on 35 USC § 112, first paragraph alleging insufficient support for every possible insertion, deletion or substitution of one or more amino acids. Although this rejection has not been raised for the newly presented claims, it is discussed insofar as it might apply. Applicants respectfully traverse this rejection.

Applicants would like to begin by noting that the Examiner stated in Paper No. 14, at page 2 that the specification is "enabled for a SPE-C mutant with specifically named amino acid substitutions in a Beta barrel of the B-subunit or a N-terminal alpha helix". Claims 35-45 represent an even narrower scope of claims than is represented by this characterization and therefore, Applicants assert that these claims are certainly in condition for allowance, having had the remainder of the rejections withdrawn therefrom, and notification to this effect is earnestly solicited.

Although Applicants strenuously believe that it is unnecessary to specifically call out substitution sites in order for the claims to be supported, such sites have been incorporated into

claims 24 through 34 and claims 35 through 44 in order to advance the prosecution of this application. General support for substitution positions even broader than that detailed by the newly presented claims are discussed at length in the specification for example at pages 11 through 14. Further, each of the positions in the newly presented claims are specifically called out in the present specification as preferred locations for substitutions. For example, support for the aspartic acid-12, tyrosine-15, and tyrosine-17 positions can be found at least at page 13, ll. 18-24.

Having either defined a specific area, or as the newly presented claims do, specific positions for substitution, the specification is replete with information and guidance regarding which amino acid should be substituted therein. Generally speaking, the amino acid to be substituted at a location is selected to include a structural change that can affect biological activity as compared with the amino acid at that location in the wild type SPE-C (pg. 9, ll. 17-20). Such substitutions may be conservative or nonconservative (pg. 9, l. 20). The specification provides a number of specifically exemplified substitutions that results in a structural change that can affect biological activity.

The first such substitution is from one type of charged amino acid to another (pg. 9, l. 22). Specific examples of this type of change can be seen in Table 7, in which lysine at position 135 is changed to aspartic acid, and lysine at position 138 is changed to aspartic acid (pg. 39, table 7). This represents a change from a positively charged amino acid to a negatively charged amino acid.

The second type of substitution that is specified is to change from a charged amino acid to a noncharged amino acid (pg. 9, ll. 22-23). A specific example of this type of substitution is given in Table 7, at page 9. In this example, aspartic acid at position 12 was replaced with alanine (pg. 39, table 7). This represents a change from a negatively charged amino acid to a noncharged amino acid. Table 7 offers another specific example of this type of change in which histidine at position 35 is changed to alanine (pg. 39, table 7). This represents a change from a positively charged amino acid to a noncharged amino acid. Yet another example is provided by changing aspartic acid at position 142 to asparagine (pg. 39, table 7), a change from a negatively charged amino acid to a noncharged amino acid.

The third broad class of substitutions that can cause structural change is substitutions in which cysteine residues are changed, resulting in the formation of disulfide bonds (pg. 9, l. 23).

The fourth type of substitution specifically stated as a way of making structural changes are those in which substitutions of amino acids result in a change in hydrophobicity. For example, changing tyrosine at position 15 to alanine, or tyrosine at position 17 to alanine (Decl. of Schlievert, p. 5), are examples of changing from hydrophillic amino acids to hydrophobic amino acids. Changing aspartic acid at position 12 to alanine, histidine at position 35 to alanine and tyrosine at position 139 to alanine (pg. 39, table 7) are also illustrative examples of changing from hydrophillic amino acid residues to hydrophobic residues.

The fifth type of substitution that can afford a structural change is a substitution that changes the size of an amino acid residue (pg. 9, l. 25). Examples of this tactic can be seen in a number of specific mutants. For example, by changing tyrosine at position 15 to serine (Decl. of Schlievert, p. 3), tyrosine at position 17 to serine (Decl. of Schlievert, p. 3), tyrosine at position 15 to alanine (Decl. of Schlievert, p. 5), tyrosine at position 17 to alanine (Decl. of Schlievert, p. 5), aspartic acid at position 12 to alanine (pg. 39, table 7), histidine at position 35 to alanine (pg. 39, table 7), lysine at position 135 to aspartic acid (pg. 39, table 7), lysine at position 138 to aspartic acid (pg. 39, table 7), and tyrosine at position 139 to alanine (pg. 39, table 7).

The sixth type of substitution that can be used to cause a structural change is to change to a conformationally restrictive amino acid or analog thereof.

The seventh type of substitution that is specifically stated as one that can cause a structural change that can affect biological activity is to change to a non-naturally occurring amino acid or analog.

The Examiner also alleges that the specification does not provide substantive evidence that the claimed vaccines are capable of inducing protective immunity. Applicants respectfully disagree with the Examiner with regard to this point. The double mutants Y15A/N38A and Y17A/N38A were prepared in Example 5 and tested for capacity to enhance endotoxin shock in Example 6. After giving a group of rabbits either a mutant SPE-C toxin or SPE-C wild type toxin, the animals were challenged with *Salmonella typhimurium* endotoxin. The rabbits that had been given mutant SPE-C toxin survived whereas those that had received the wild-type toxin died (see Table 4 on page 37).

Further studies involved immunization of rabbit groups with 2 weekly doses of SPE-C double mutants. Blood samples before and after immunization were compared for antibodies

against streptococcal derived wild type SPE-A. The blood samples after immunization had higher levels of antibodies (see Table 5 on page 38).

The immunized animals were then challenged with wild type SPE-C and then 4 hours later with *Salmonella typhimurium* endotoxin. None of the rabbits that were immunized died whereas rabbits that were not immunized died (see Table 6 on page 39).

Applicants would also like to address the Examiner's comments regarding stability of the mutants. The Examiner alleges that the specification does not provide guidance on how multiple amino acids can be deleted, substituted or inserted for the production of a stable protein. The Applicants respectfully disagree regarding the relevancy of protein stability for this invention. As pointed out in the response to the first office action, the critical issue for a mutant to function as a vaccine is nonlethality rather than stability. The protein does not have to remain intact to function as a vaccine. There is support for claims that the mutants can be used as vaccines. Two double mutants (Y15A/N38A and Y17A/N38A) were prepared as described in Example 5 and then evaluated in Example 6. The mutations were effective as vaccines. Finally, there are no claims in the present invention regarding the stability of the mutations.

By describing the discrete residues and specific structural features of the protein that are suitable for making mutations that yield a nonlethal SPE-C as well as showing specific examples of mutations that caused immunization against wild type SPE C and endotoxin, Applicants have exceeded the standard for enablement under § 112, first paragraph.

Applicants are looking forward to the Examiner's acknowledgment that the newly presented claims are enabled and comply with § 112 first paragraph, and that they are allowable.

35 USC § 112, Second Paragraph Rejections

The Examiner rejected claim 10 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner alleges that recitation of "substantially enhance endotoxin shock" in claim 10 is vague. Applicants have deleted this phrase from the newly presented claims, and therefore believe that this rejection is no longer applicable, and should therefore be withdrawn.

Summary

In summary, Applicants assert each of the newly presented claims 24-44 are in condition for allowance, and notification of that effect is earnestly solicited. The Examiner is invited to contact Applicants' undersigned representative at the telephone number provided below, if the Examiner believes that prosecution of the application can be expedited thereby.

Respectfully submitted,

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Date: Aug 8, 2001

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